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The Effect of Manure and the Bioaccumulation of Heavy Metals Hg, Cu and Zn at Ex-Gold Mining Land on the Growth of Silk Tree (*Paraserinthes falcataria* (*L.*) Nielsen) in Bombana, Southeast Sulawesi

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Abstract

One of the negative effects of gold mining activity is soil contamination by heavy metal. The aims of this study were two folds; to assess the effect of manures (cow, goat, chicken) (i) on the growth of silk tree (*Paraserianthes falcataria* (L.) Nielsen) and (ii) on the bioaccumulation of heavy metals (Hg, Cu, Zn) in the plant organs of the silk tree planted in the ex-gold mining land in Bombana. This is an experimental research design with a completely randomized design consisting of one control (without manure) and 4 treatments (cow, goat and chicken manures) with 5 repplicates. The plant growth parameters observed are plant height, stem diameter, number of leaves, leaf area, and metal content of Hg, Zn, and Cu in plant organs. ANOVA with Duncan Multiple Range Test (DMRT) were used for the analysis. The results showed that the treatment with manure has an impact on the parameter of plant growth and bioaccumulation of Hg, Cu and Zn in the plant organs. DMRT showed that the treatment with manures has a significant influence on the growth and bioaccumulation of Hg, Cu, and Zn in the plants. The manure with cows was better in supporting growth of silk tree compared to those with chicken, goat as well as control. Treatment with manure reduced bioaccumulation of Hg but increased bioaccumulation of Cu and Zn in the plant organs. The bioaccumulation of Hg, Cu and Zn was found the highest in the root than in the stem and leaves. The total bioaccumulation of Zn in plant organs was the highest compared with that of Cu and Hg.

Keyword: Manure, Growth, Heavy Metals, Silk Tree, Ex-Gold Mining Land

1. Inroduction

Mining industry always received a great attention as it has high risk on environment. It is often found that mining practices without good planning have caused great environmental damage [38]. Improper handling of mining land has caused the government to spend much more to restore the ecological, social and economical function of the environment than the benefits received [1, 5, 7, 10].

The ex-mining land is often left unattended and unplanted in which this have caused the land degraded, unproductive, and becoming marginal. If not managed properly, it will decrease, for example, air quality due to dust, water quality (physic and chemical qualities) due to erosion and sedimentation, groundwater quantity and quality due to chemical pollution, the shift of land use (change of topography, drainage, and vegetation), and the changing shape of the land surface due to overburden piles. As a result of gold mining, chemical, physical and biological damage can occur [8]. For example, the pollution of soil, water and vegetation occurs due to the use of Hg (mercury) in leaching. The high concentration of Cu and Zn was also detected on gold mining land in Bombana. Such chemical content can be hazardous to the plant [8, 13]. The decrease of Cu levels in soil solution has increased pH in soil as attached to the soil matrix. The level of Zn in soil is higher at low pH than at high pH whereas the solubility of Zn is higher in high acidity than in low acidity [2].

The deficiency of essential nutrients such as nitrogen and phosphorus, the soil acidity (low pH) and mineral toxicity which are mainly due to heavy metals such as mercury (Hg), copper (Cu) and zinc (Zn), are common and as the major obstacles encountered in the ex-mining land [9], such as in the ex-gold mining land in Bombana.

The extreme conditions of mining land can be handled in two ways: *first*, the improvement of soil conditions with ameliorant engineering applications, for example, the user of organic and unorganic fertilizer to support the plant growth; the *second* alternative is to choose plants that can adapt to such extreme conditions [3].

In this research, the use of organic manure on ex-mining land, being present abundant in Bombana, is studied. After being processed into organic fertilizer, the organic manure was applied to the poor-nutrient ex-mining land. The manure will support the availability of nutrients and improve the physical, chemical and biological soil properties [9]. *Sengon* (Paraserianthes falcataria (L.) Nielsen) was used as agents of phytoremediation to restore the ex-mining land with heavy metal contamination as the plants can grow in poor, dry, muddy or slightly salty soil [4].

2. Method

Material

The materials used in this study consist of; (i) sample of ex-mining soil, to be analyzed its physicochemical properties and used it as a growing medium for plant bioaccumulator screening; (ii) silk trees (*Paraserianthes falcataria* (L.) Nielsen); (iii) polybags with size of 12 x 8 cm as container screening; (iv) water, for watering plants during screening; (v) Chemical compounds, used for the analysis of physicochemical properties of heavy metal levels, i.e., Hg, Cu and Zn, in the ex-gold mining soil and in the plant organs.

Tools

A number of tools were used in this study such as; shovels, hoes, soil sieve and the sack for taking ex-gold mining soil samples; stationery and camera for the documentation; scales for weighting the ex-gold mining soil samples and plant organs; multi tester (pH meter, Higro meters), Lux meter; GPS used for determining the coordinates of a former quarry soil sampling; oven, filter paper, glass-beaker, flask peck, Microwave oven, AAS, Computer as a data processor.

Procedure

This is an experimental research with a completely randomized design (CRD). There are three units of animal manure treatment; i.e., crib, goat, chicken manure, and one control unit or without manure, with five repetitions for each unit, in total there are 20 units in the greenhouse experiments.

The procedure consisted of several steps; (i) conducting survey on ex-gold mining sites in Bombana Regency; (ii) carring out analysis of soil sampling in terms of physical and chemical properties of the soil character and as a growing medium; (iii) preparing the growing plant media with the treatment of manure (crib, goat, chicken) with the same dose of 150g/10kg soil, equivalent to 30 tonnes/ha. In addition to animal manure, the treatment for all growing media was to put the same basic fertilizer; i.e., urea (1,5g/kg soil is equivalent to 300kg/ha), SP-36 (1g/10kg of soil equivalent to 200kg/ha) and KCL (0,5 g/kg soil is equivalent to 100kg/ha) as well as the addition of lime $CaCO_3$ as much as 10g/10kg of soil equal to 2 ton ha^{-1} (Harry, 1994). Furthermore, bioaccumulator screening of silk tree (*Paraserianthes falcataria* (L.) was grown in the green house; plant maintenance such as watering is done daily and measure growth parameters every two weeks; analyze plant growth (height, stem diameter, number of leaf including leaf area) and bioaccumulation of Hg, Cu, Zn in plant organs.

The data relating to the growth and the concentration of heavy metals (Hg, Cu, Zn) was obtained from the measurements using AAS tool for the entire sample in the test ANOVA (analysis of variance) in SPSS software. The corelation between the heavy metal concentrations in the parts of the plant and those in the soil was analyzed with Duncan Multiple Range Test (DMRT). The correlation between two factors is statistically significant if p-value is less than 0.05 ($p<0.05$).

3. Result and Discussion

The results showed that the treatment with cow manure, goat manure and chicken manure significantly affect on the growth and the bioaccumulation of heavy metals Hg, Cu, and Zn in the plant organs silk tree (*Paraserianthes falcataria* (L.) Nielsen) grown on ex-gold mining land. For more specific, the results of each parameter observed are described as follows:

a. Plant Height

The average plant height on the silk tree (*Paraserianthes falcataria* (L.) Nielsen) without manure treatment (control) and with manure (crib, goat, chicken) can be seen in Table 1.

Tablel 1. Plant Height of Silk Tree (*Paraserianthes falcataria* (L.) Nielsen) (cm)

P	Plant Height **(cm) in 0 - 12 Weeks**							**Means**
	0	**2**	**4**	**6**	**8**	**10**	**12**	
C	13,34	14,28	16,38	19,84	20,42	22,17	24,56	**18,57a**
S	15,99	23,84	29,24	34,44	41,64	47,08	50,82	**35,29b**
K	15,14	17,56	20,18	27,06	28,44	30,2	33,5	**22,58ab**
A	14,46	17,84	24,01	29,32	33,04	38,17	40,02	**28,12ab**

Two means followed by the same letters in the same column indicate they are not significantly different (p=0.05). P = Animal manure Treatments: C = Control, S = Cow, K = Goat and A = Chicken.

The addition of organic matter (manure) into the soil has resulted in a positive response to the plant growth. This study showed that the treatment with manure as organic material supplied to the plant growing medium resulted in better growth on the silk tree (*Paraserianthes falcataria* (L.) Nielsen) than those planted on the medium without treatment of animal manure.

The results also showed that the treatment with different manure gave the different effect on the plant growth of silk tree (*Paraserianthes falcataria* (L.) Nielsen) even though the same dose of each manure was applied to the treatment units, that was 150g/10kg. The study showed that the average plant height silk tree (*Paraserianthes falcataria* (L.) Nielsen) planted in the ex-gold mining soil media was as follows; in the media with the treatment of cow manure (35.29 cm), henhouse manure (28.12 cm), goat manure (22.58 cm) and controls (18.57 cm). The average height of silk tree planted in the media with the treatment of cow manure was significantly different from the height in the controls but it was not significantly different with the height in chicken manure and manure goat.

The significant difference on the silk tree growth between the treatment with and without manure is an evidence that manure is very helpful in providing essential macro and micro nutrients useful to support the plant growth.

The composition of nutrition contained in cow manure is best suited to support the hieght of sengon plants. The content of NPK element in the cow manure on the land, applied to ex-gold mining soil, is used as a medium to grow plants sengon (Paraserianthes falcataria (L.) Nielsen) which consists of N 0.58%, 12.67% P, K 0.53%; Goat manure N 0.41%, 11.24% P, K 0.31%; and chicken manure, i. e., 0.37% of N, P and K 11.35% 0.36% [35]. From the composition of macro nutrients NPK, it can be seen that the content of the cow manure is higher than the other fertilizers in which the plant growth of silk tree (*Paraserianthes falcataria* (L.) Nielsen) with cow manure is better than the plant growth on goat and chicken manures. This is clearly shown in plant height charts and graphs in which there is a relatively high growth rate on sengon plants planted in the ground ex-gold mining land with cow manure until the age of 12 weeks after planting, as depicted in Figures 1 and 2.

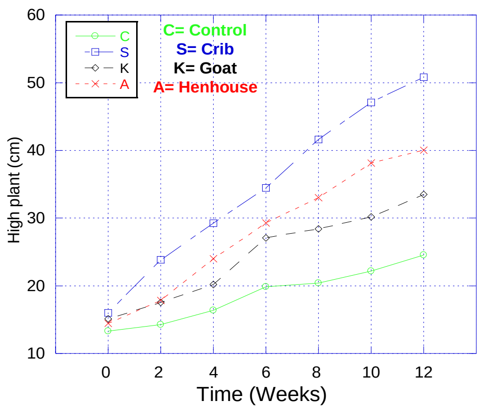


Figure1. Graph of Plant Height (cm) 0-12 weeks

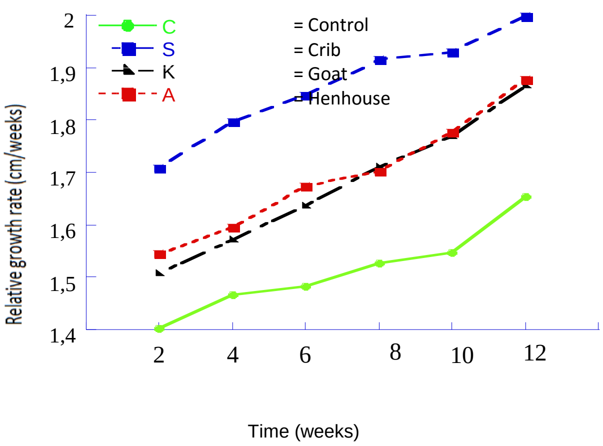


Figure 2. Relative growth rate of plant height (cm) 0-12 weeks

Based on the analysis of plant height increment for difference manure as in Table 1, it indicates that the treatment of cow (crib) manure. The biggest difference on the plant height increment with cow treatment occurs at week 8, that is around 7.2 cm, with goat manure treatment at week 6 with 6,66 cm and with chicken (hen house) manure treatment at week 4 with 5.31 cm. The high increment on the plant growth with goat and chicken manure occurs in the early growth of plants which is due to the fact that the goat and henhouse manure is a fertilizer with quick microorganism heat and therefore its availability in the planting medium becomes quickly exhausted. While the highest plant height increment in the treatment of cow manure occurs at the end of plant growth, due to the fact that cow manure is a cold fertilizer and the supply of available nutrients to the plants takes longer. Thus, the dvantages of cold manure nutrients are that they are present in the cow manure for longer period of time in the plant growth media.

b. Stem Diameters

The observation of the average stem diameters of the silk tree (*Paraserianthes falcataria* (L.) Nielsen) without (control) and with manure treatment (crib, goat and henhouse) can be seen in Table 2.

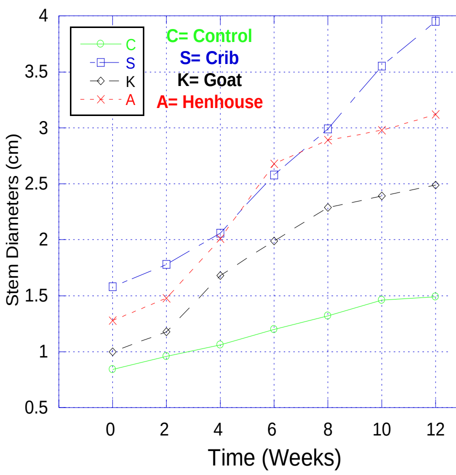
Table 2. Stem Diameter of Silk Tree (*Paraserianthes falcataria* (L.) Nielsen) (cm)

P	**Stem Diameter (cm) in 0 - 12 Weeks**							**Means**
	0	**2**	**4**	**6**	**8**	**10**	**12**	
C	0,84	0,96	1,06	1,2	1,32	1,46	1,49	**1,19a**
S	1,58	1,78	2,06	2,58	2,99	3,55	3,95	**2,64b**
K	1	1,18	1,68	1,99	2,29	2,39	2,49	**1,86a**
A	1,28	1,48	2,01	2,68	2,89	2,98	3,12	**2,35b**

Means followed by the same letters, in same column was not significantly different (p=0.05). P= Animal manure; C=Control, S= Cow, K= Goat, A= Chicken.

The treatment of manure is also a significant effect on the increase in stem diameter of plants silk tree (*Paraserianthes falcataria* (L.) Nielsen). DMRT results showed that the increase of stem diameter in the silk tree plants with the treatment of cow manure is significantly different from those with the goat manure treatment and control but it is not different from those with henhouse manure treatment. The results showed that the largest growth in diameter 2.6 cm for the cow manure, 2.35 cm for the henhouse manure, 1.86 cm for goat manure and the lowest is 1.19 cm for controls. The lower in the magnitude of the diameter for the treatment with cow manure is presumably because the element of K (potassium) in the cow manure is greater than K elements being present in goat and henhouse manure. This element K is used for secondary cell division by meristematic activities of stem cells which tend to widen and effect on stem diameter and biomass.

Relative growth rate of the largest diameter silk tree plant with cow manure treatment is 0,507cm/2 weeks and lowest is in the control, that is 0,168cm/2 weeks. Based on the analysis of variance (ANOVA), it showed that the manure treatment significantly influence the control where the p-value is less than 0.05. This indicates that the use of manure can increase the growth rate relative diameter stem of the plant. The silk tree stem diameter and its relative growth rate can be seen in Figures 3 and Figure 4.



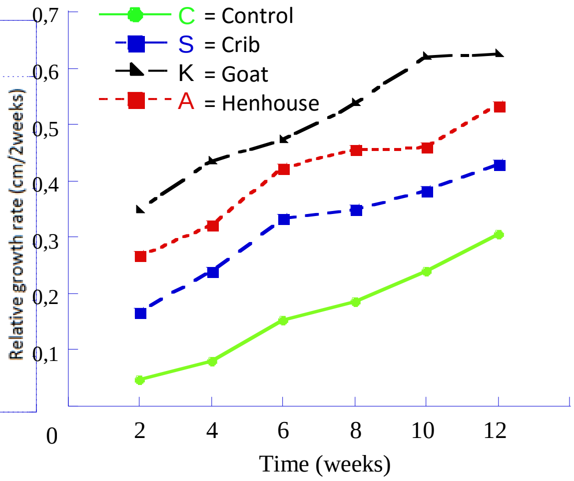


Figure 3. Graph of stem diameter of silk tree (*Paraserianthes falcataria* (L.) Nielsen) (cm)

Figure 4. Relative growth rate of stem diameter of silk tree (*Paraserianthes flacataria* (L.) Nielsen) (cm)

c. Leaf Area and Number of Plant Leaves

The observation of the average number of silk tree (*Paraserianthes falcataria* (L.) Nielsen) plant leaves without (control) and with manure (cow, goat and chicken) can be seen in Table 3.

Table 3. Number of Plant Leaves on Silk Tree (*Paraserianthes falcataria* (L.) Nielsen)

P	Number of Plant Leaves (Pieces)							Means
	0	2	4	6	8	10	12	
C	2,4	2,6	3,1	3,5	4,4	4,8	5,2	**3,71a**
S	3	3,8	5	6,9	8,2	9,22	10,27	**6,63b**
K	2,1	2,6	4,6	4,91	5,03	5,09	6,03	**4,04ab**
A	2,2	2,91	5,09	6,01	6,99	7,06	7,89	**5,45b**

Means followed by the same letters, in same column was not significantly different (p=0.05). P= Animal manure; C=Control, S= Cow, K= Goat, A= Chicken.

Based on Table 3 shown the average number of leaves (pieces) silk tree (*Paraserianthes falcataria* (L.) Nielsen) more on the treatment of cow manure (6,33 strands), henhouse manure (5.45 strands), goat manure (strands 4.04) and the lowest number of leaves on the control (3.71 strands). Based on analysis of variance (ANOVA) showed that manure application has a significant effect on the number of plant leaves sengon (p-value <0.05) and a further test results DMRT showed that the average number of leaves with cow manure is significantly different from that for the control but not significantly different from that with henhouse manure. Based on ANOVA statistical test and DMRT results, they showed that the leaf area (cm^2) also showed a similar result to the number of leaves on silk tree, as shown in Table 4 and Figure 5.

Table 4. Leaf Area (Cm^2) of Silk Tree (*Paraserianthes falcataria* (L.) Nielsen)

P	**Leaf Area (cm^2)**							**Means**
	0	**2**	**4**	**6**	**8**	**10**	**12**	
C	6,57	7,38	8,28	8,62	9,03	10,22	13,21	**9,04a**
S	9,78	10,37	10,74	16,11	21,68	27,23	32,25	**18,31b**
K	9,15	9,37	14,63	15,22	17,73	18,83	20,26	**13,03ab**
A	9,62	10,05	16,28	18,83	20,2	22,49	23,88	**17,34b**

Means followed by the same letters, in same column was not significantly different (p=0.05). P= Animal manureTreatments: C= Control, S= Cow, K= Goat, A= Chicken.

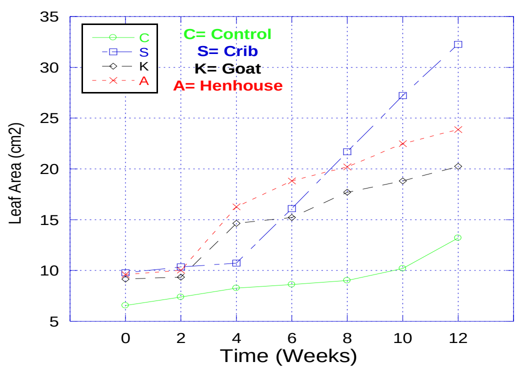


Figure 5. Graph of leaf area (cm^2)

d. Percentage and bioaccumulation of Heavy Metal in Plant Organs

The percentage of metal bioaccumulation of Hg, Cu, and Zn presented in the plant organs; roots, stems and leaves of plants, both on the control (without manure) and on the manure treatment (cow, goat, and chicken) can be seen in Figure 6-8.

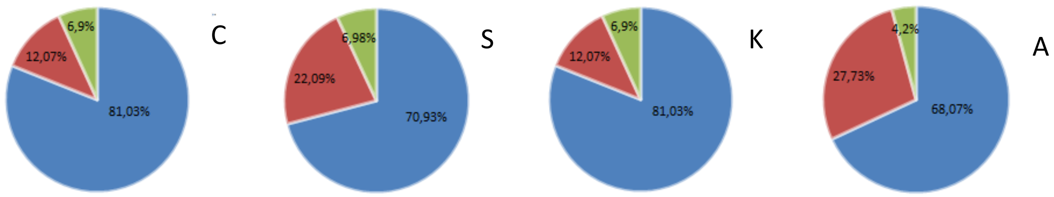


Figure 6. Diagram of Hg bioaccumulation on plant organs that translocate to leaf, stems and roots after 12 weeks planting. | Stems |, | Leaves |, | Roots |

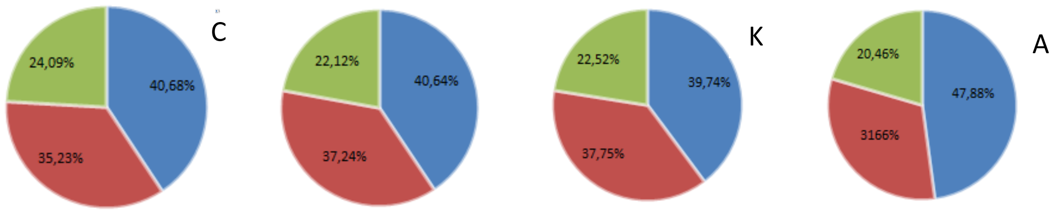


Figure 7. Diagram of Cu bioaccumulation on plant organs that translocate to leaf, stems and roots after 12 weeks planting. | Stems |, | Leaves |, | Roots |

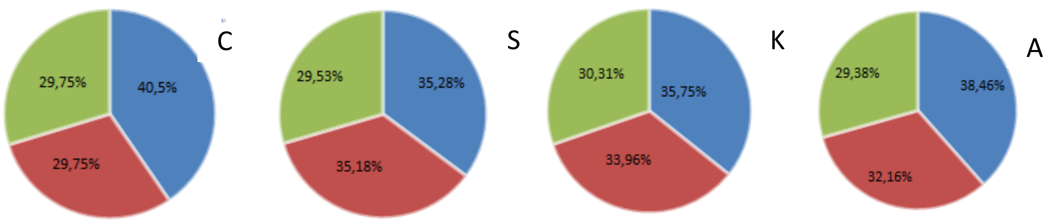


Figure 8. Diagram of Zn bioaccumulation on plant organs that translocate to leaf, stems and roots after 12 weeks planting | Stems |, | Leaves |, | Roots |

Total bioaccumulation of Hg, Cu and Zn in plant organs of silk tree on the control (without manure) and with manure treatment (crib, goat and henhouse) can be seen in Table 5-7.

Table 5. Bioaccumulation of Hg in plant organs silk tree (ppm)

P	Soil		Bioaccumulation of Hg (ppm)			Total in Plant Organs
	Before	After	Roots	Stems	Leaves	
C	6,5	0,0201a	0,0094a	0,0043a	0,0007a	**0,0144**
S		0,0460b	0,0061b	0,0019b	0,0006a	**0,0086**
K		0,0463b	0,0047b	0,0007b	0,0004a	**0,0058**
A		0,0399b	0,0081b	0,0033b	0,0005a	**0,0119**

Means followed by the same letters, in same column was not significantly different (p=0.05). P= Animal manure; C= Control, S= Cow, K= Goat, A= Chicken.

Table 6. Bioaccumulation of Cu in plant organs silk tree (ppm)

P	Soil		Bioaccumulation of Cu (ppm)			Total in Plant organs
	Before	After	Roots	Stems	Leaves	
C	644,79	0,3051a	0,1449a	0,1255a	0,0858a	**0,3562**
S		0,4133a	0,2082a	0,1908a	0,1133b	**0,5123**
K		0,4592a	0,1837b	0,1745b	0,1041b	**0,4623**
A		0,2118a	0,3010b	0,1990b	0,1286b	**0,6286**

Means followed by the same letters, in same column was not significantly different (p=0.05). P = Animal manure; C= Control, S= Cow, K= Goat, A= Chicken.

Table 7. Bioaccumulation of Zn in plant organs silk tree (ppm)

P	Soil		Bioaccumulation of Cu (ppm)			Total in Plant organs
	Before	After	Roots	Stems	Leaves	
C	360	1,6080a	0,8148a	0,5986a	0,5986a	**2,012**
S		1,7852a	0,8161a	0,8184a	0,6850b	**2,320**
K		1,7573a	0,8715a	0,8282b	0,7393b	**2,439**
A		1,6693a	0,8237a	0,6889a	0,6293b	**2,142**

Means followed by the same letters, in same column was not significantly different (p=0.05). P= Animal manure; C= Control, S= Cow, K= Goat, A= Chicken.

The results from metal content analysis indicate that the bioaccumulation of Hg, Cu and Zn were present in the supreme organ of the plant from the roots to stems and leaves. The difference is due to bioaccumulation. According to [6], the absorption and accumulation of heavy metals in plants can be divided into three continuous process, i.e., metal uptaking by the root, metal translocating from the roots to other plant parts and metal localizing on specific organs to maintain order but not to inhibit the metabolism of the plant.

Bioaccumulation of Hg, Cu and Zn in the root organ due to the soil media has a high metal content and the roots are muselec organ that can bind heavy metals being present in the root. This is the reason that the metal is present more in the roots than other plant organs. After the metal is transported into the root organ, it is then transported, via the transport tissue, namely, xylem and phloem, to the other plant organs. To improve the efficiency of transport, metal is bound by the chelate molecules. According to [14], the absorption amount of heavy metals in plant roots can also be due to the process lignification of the root and the cessation of metal transport toward the leaves so that the metal accumulates in the roots.

Based on the results of heavy metals (Hg, Cu and Zn) in silk tree (*Paraserianthes falcataria* (L.) Nielsen) grown on ex-gold mining land, it showed that metal accumulation in plant organs at the age of 3 months is, from the highest to the lowest concentration, Zn, Cu and Hg, respectively. This is because the percentage of Zn nutrients needed by plants to grow is higher than the percentage of metal elements, Cu and Hg, respectively, despite the presence of Hg is more on the ground [14].

The ccumulation of heavy metal Zn in the silk tree organs is higher than that of Cu and Hg. This is because the metal Zn is an essential element for the growth of all kinds of animals and plants. Zn is found virtually in all cells and it is a very important element for the growth of human, animal or plant after Fe. Zn role in catalytic reaction, i.e., almost 100 types of enzymes with the ability catalyst in the reaction, depends on Zn. Zinc also plays an important role in developing and stabilizing the protein structure of the cell membrane structure and it is also as a catalyst enzyme superoxide (CuZnSOD) [11].

5. Conclusion

It was shown that animal manure has a significant influence on the growth of silk trees planted in the ex-gold mining land of Bombana Southeast Sulawesi. Moreover, it was found that the cow manure has the best effect in increasing the plant height, stem diameters, leaf area, the number of leaves and the root length.

The highest bioaccumulation percentage of heavy metal; Hg, Cu and Zn, was found in roots compared those found on stems and leaves of silk tree organs (*Paraserianthes falcataria* (L.) Nielsen). Total bioaccumulation of Hg in the plant organs was higher on the control group than on the manures treatment. The highest bioaccumulation of Cu was found on the tree with chicken manure, whereas the highest bioaccumulation of Zn was in the trees with goat manure. The higher accumulation of heavy metals found in the silk tree organs was Zn compared to the accumulation of Cu and Hg.

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